JOURNAL OF VIROLOGY, Feb. 1985, p. 658–666
0022-538X/85/020658-09$02.00/0

Vol. 53, No. 2

Human Glioblastoma Cells Persistently Infected with Simian Virus 40 Carry Nondefective Episomal Viral DNA and Acquire the Transformed Phenotype and Numerous Chromosomal Abnormalities

LEONARD C. NORKIN,* VALERIE I. STEINBERG, AND MAGDALENA KOSZ-VNENCHAK
Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003

Accepted 5 July 1984/Accepted 10 October 1984

A stable, persistent infection of A172 human glioblastoma cells with simian virus 40 (SV40) was readily established after infection at an input of 450 PFU per cell. Only 11% of the cells were initially susceptible to SV40, as shown by indirect immunofluorescent staining for the SV40 T antigen at 48 h. However, all cells produced T antigen by week 11. In contrast, viral capsid proteins were made in only about 1% of the cells in the established carrier system. Weekly viral yields ranged between 10^4 and 10^6 PFU/ml. Most of the capsid protein-producing cells contained enormous aberrant (lobulated or multiple) nuclei. Persistent viral DNA appeared in an episomal or "free" state exclusively in Southern blots and was indistinguishable from standard SV40 DNA by restriction analysis. Viral autointerference activity was not detected, and yield reduction assays did not indicate defective interfering particle activity, further implying that variant viruses were not a factor in this carrier system. Interferon was also not a factor in the system, as shown by direct challenge with vesicular stomatitis virus. Persistent infection resulted in cellular growth changes (enhanced saturation density and plating efficiency) characteristic of SV40 transformation. Persistent infection also led to an increased frequency of cytogenetic effects. These included sister chromatid exchanges, a variety of chromosomal abnormalities (ring chromosomes, acentric fragments, breaks, and gaps), and an increase in the chromosome number. Nevertheless, the persistently infected cells continued to display a bipolar glial cell-like morphology with extensive process extension and intercellular contacts.

The papovaviruses have attracted medical interest primarily because of their ability to induce tumors in laboratory animals and neoplastic transformation of cells in culture. Yet despite the wide distribution of these viruses in populations of their natural hosts (including humans), they have not been associated with any common human illness. However, papovaviruses are the etiological agents of progressive multifocal leukoencephalopathy (PML), a rare, slowly progressing demyelinating disease, found predominantly in patients with impaired cell-mediated immunity (54; see reference 38 for a review). JC virus, a human papovavirus, has been implicated in most cases of PML, and simian virus 40 (SV40) has been associated with several others (52). There were no clinical differences between the JC virus- and SV40-associated cases. Also note that a disease with striking similarities to PML occurs in the Asian rhesus macaque (13), the natural host of SV40.

Primary papovaviral infections, which are either aclinical or very mild, often lead to the persistence of latent virus in several organ systems for indefinite periods (12, 15, 16). It is not known whether PML results from activation of latent papovaviral infections within the brain or, instead, from viruses activated elsewhere in the body which spread to the brain. Regardless, the brain appears to be the only organ in which papovaviral infection is pathogenic.

There is no animal model of PML, since it has not yet been possible to experimentally induce this disease in animals. Also, the development of cell culture model systems of PML has been seriously impeded by the lack of easily available cell cultures able to support efficient JC virus replication and able to remain viable for extended periods of time (39). However, because SV40 has been associated with PML in humans (52), the study of the interaction of this readily cultivable virus with human neural cells in vitro should provide insights into the pathogenesis of PML.

In this report we describe a system of human glioblastoma cells (A172 line) persistently infected with SV40 which reflected several aspects of PML in the human brain. Among the features of this system were the following. Whereas the SV40 tumor (T) antigen was produced by all cells of the system, only a small percentage of the cells produced virus. The virus-producing cells contained grossly swollen aberrant nuclei. Viral DNA was nondefective and was found to persist exclusively in an episomal or "free" state. The cells displayed growth changes (i.e., increased saturation density and plating efficiency) characteristic of SV40 transformation. Persistent infection also caused an increase in the number of cellular chromosomes and in the frequency of cytogenetic damage. The implications of these findings are discussed.

MATERIALS AND METHODS

Cells and virus. The A172 line of human glioblastoma cells (11) was kindly provided by Frank O'Neill. Cells were cultivated in Dulbecco modified Eagle (DME) medium supplemented with 10% fetal calf serum in a humidified 5% CO_2 atmosphere. SV40 strain 777 was prepared for infection as previously described (36).

Indirect immunofluorescence. SV40 T and V antigen-producing cells were identified by indirect immunofluorescent staining as previously described (36). Antiserum prepared by inoculation of rabbits with purified SV40 empty capsids previously disrupted by treatment with sodium dodecyl sulfate was kindly provided by Harvey Ozer. Antisera prepared in rabbits against the purified SV40 capsid proteins VP1 and VP3 were kindly provided by Harumi Kasamatsu.

* Corresponding author.

DNA preparation and analysis. Viral and cellular DNAs were selectively extracted by the method of Hirt (17). Viral DNA-containing Hirt supernatant fractions were concentrated with polyvinylpyrrolidone, and form I supercoiled DNA was isolated by equilibrium centrifugation in CsCl-ethidium bromide gradients followed by dialysis against 10 mM Tris–1.0 mM EDTA (pH 7.5). Hirt precipitate fractions, which contained high-molecular-weight cellular DNA, were suspended in Dulbecco phosphate-buffered saline (137 mM NaCl, 8 mM $Na_2HPO_4 \cdot 7H_2O$, 1.5 mM KH_2PO_4, 3 mM KCl) and phenol extracted as described by Ketner and Kelly (21). DNA was precipitated with ethanol and dissolved in 10 mM Tris–1.0 mM EDTA (pH 7.5).

Restriction endonucleases were obtained from Bethesda Research Laboratories, and reactions were carried out with a 4- to 10-fold excess of enzyme under the conditions recommended for each enzyme by the manufacturer. Restriction fragments were separated by electrophoresis in 1.0% agarose gels in a Tris-acetate buffer (50 mM Tris, 20 mM sodium acetate, 2 mM EDTA, 18 mM NaCl [pH 8.05]). Gels were stained by immersion in ethidium bromide (0.5 μg/ml) for 30 min and viewed with a shortwave UV transilluminator.

Restriction fragments were transferred from the gels to nitrocellulose membrane filters (0.45 μm; Schleicher & Schuell, Inc.) in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) by the blotting method of Southern (49). The filters were then air dried, baked in vacuo for 2 h at 80°C, and then incubated for 4 h at 68°C in heat-sealed plastic bags containing prehybridization solution (6× SSC, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin). The prehybridization solution was then replaced with hybridization solution (6× SSC, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.5% sodium dodecyl sulfate, 0.001 M EDTA, sheared denatured calf thymus DNA [100 μg/ml]), to which 100 ng of SV40 [^{32}P]DNA (specific activity, 10^5 cpm/ng) was added. SV40 [^{32}P]DNA was prepared from a pBR322 plasmid (p777B) that contained the SV40 genome (strain 777) cloned into the *Bam*HI site (kindly provided by Michael Piatak) and labeled in vitro by nick translation (45).

Hybridization was carried out for 18 to 24 h at 68°C, the hybridization solution was removed, and the filters were washed twice for 0.5 h at 68°C in 2× SSC–0.5% SDS, once for 0.5 h at 68°C in 2× SSC, and once for 0.5 h at room temperature in 2× SSC. The filters were then air dried, and viral DNA-containing bands were detected by autoradiography with Kodak XAR X-ray film with or without intensifying screens.

Transformation assay. Monolayer cultures were suspended with trypsin, washed, serially diluted in DME medium containing either 1, 2.5, or 10% fetal calf serum, and plated at several cell concentrations ranging from 1.6×10^3 to 1.3×10^5 cells per 35-mm plastic dish in DME medium containing either 1, 2.5, or 10% fetal calf serum. Plates were incubated at 37°C. Media were changed on day 6. On day 10, media were removed, and the cells were washed with Hanks balanced salt solution, fixed with 95% ethanol, and stained with Giemsa.

Chromosome studies. Sister chromatid exchanges (SCE) were detected by a technique that accomplishes the differential staining of the two chromatids of a single chromosome (43). Briefly, cells were incubated with 5-bromodeoxyuridine for two rounds of cell replication, allowing its incorporation into both DNA strands in one chromatid, but in only one strand of its sister chromatid. Such chromatids stain differentially with a combination of the fluorochrome Hoechst 33258 plus Giemsa.

RESULTS

Establishment of the persistent infection. Subconfluent monolayer cultures of the A172 line of human glioblastoma cells were infected with SV40 at an input multiplicity of 450 PFU per cell. Only 11% of the cells were initially infected, as indicated by indirect immunofluorescent staining for the SV40 T antigen at 48 h postinfection. In contrast, 92% of CV-1 green monkey kidney cells, infected under the same conditions, displayed the SV40 T antigen at 48 h.

After the first week of infection, when only about half of the A172 cells remained attached to the culture dishes, the titer of extracellular virus in the medium and the titer of cell-associated virus harvested into an equivalent volume of medium were each about 5×10^5 PFU/ml. Parallel infected CV-1 cultures yielded about 2×10^7 PFU/ml and were essentially completely destroyed after 1 week.

The SV40-infected A172 cultures were fluid changed after the first week and thereafter did not display any noticeable cytopathic effects whatsoever, despite the continuous production of infectious virus. Indeed, the productively infected cultures were routinely passaged (1:3 subculture) on a weekly basis after the third week, despite viral titers of between 10^4 and 10^6 PFU/ml in the media.

The fraction of T-antigen-producing cells increased during subsequent weeks such that about half of the cells were T antigen positive at week 4 and nearly all appeared positive by week 11 (Fig. 1A). However, the fraction of T antigen-producing cells inexplicably fluctuated during later weeks. In contrast, only about 1% of the cells in the established SV40-A172 system produced viral structural proteins at any time, as shown by indirect immunofluorescent staining with antisera prepared against purified and subsequently disrupted empty SV40 capsids (Fig. 1B). Similar results were obtained with antisera prepared against purified SV40 capsid proteins VP1 and VP3 (Fig. 1C and D). This indicates that SV40 persistence in A172 cells is mediated by a carrier culture mechanism in which only a small fraction of the cell population supports the productive infection at any moment.

Note that some of the cells in the SV40-A172 carrier system contained capsid protein-producing nuclei that attained enormous proportions. Some of these nuclei also displayed extraordinary deformations, having become irregularly lobulated or multiple (Fig. 1B, C, and D). This nuclear aberrancy did indeed correlate with viral late gene expression, since it was displayed by 75% of the capsid protein-producing nuclei, whereas it was rare among the much larger population of T antigen-producing cells.

Physical state and nature of SV40 DNA in the carrier system. The state of the viral genomes in the persistently infected system was examined by Southern blot analysis (3, 21, 49) of samples enriched for high-molecular-weight DNA by the method of Hirt (17) (Fig. 2). The comigration of the single band of SV40 sequences in the uncleaved sample (lane 4) and the single band in the sample cleaved with *Sst*I (which does not recognize any sites on the SV40 genome; lane 5) with form I standard viral DNA (lane 2) indicates that all of the detectable viral DNA in the SV40-A172 carrier system is present in the form of free superhelical molecules. Reconstruction experiments (lane 2) indicated 5 to 10 and 0.5 to 1 viral genome equivalents per cell, on the average, at weeks 12 and 21, respectively.

The generation of single bands of SV40 sequences upon restriction with either *Eco*RI, *Bam*HI, or *Bgl*I (Fig. 2, lane 6,

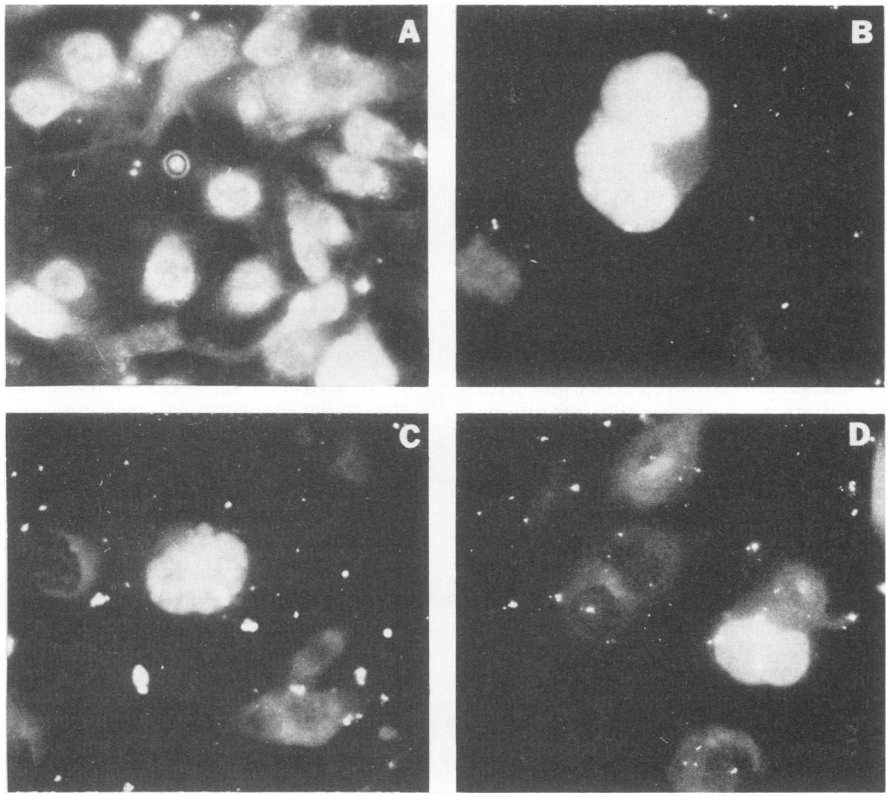

FIG. 1. Fluorescence micrographs of SV40-infected A172 cells after 11 weeks of persistent infection. Cells stained with anti-T serum (A), antiserum prepared against disrupted empty capsids (B), anti-VP1 serum (C), and anti-VP3 serum (D). All plates were photographed at the same magnification.

7, or 8, respectively), each of which recognizes a different single site on the SV40 genome, and the comigration of those bands with form III standard SV40 DNA (lane 2) shows that the viral genomes in this system do not contain the reiteration-type variants that arise in other SV40 systems of persistent infection and during serial undiluted passage of SV40 in other cell types (8, 23, 37). In particular, note the absence of variant viral genomes containing multiple origins of replication, which would have been revealed by the generation of aberrant fragments upon restriction with *Bgl*I. Variant viral genomes were also not detected upon a similar analysis of low-molecular-weight Hirt fractions (17) prepared from the same carrier system samples (lane 1).

The absence of aberrant fragments upon digestion with each of the "one-cut" enzymes suggests that the viral genomes in this system are largely, if not entirely, nondefective. This was supported by the restriction of both high- and low-molecular-weight Hirt fractions with *Hin*dIII, which recognizes six sites on the standard SV40 genome. Restriction of the carrier system samples with *Hin*dIII did not generate any detectable aberrant fragments (data not shown).

The absence of a notable fraction of viral variants was also implied by several other findings. For example, all of the PFU generated by the system produced large plaques on CV-1 monolayers, similar to those produced by the original input standard virus stock. Also, autointerference activity, indicative of a subpopulation of defective viruses, was not detected. Autointerference would have been revealed if there were an inverse relationship between the input multiplicity of infection and the viral yields obtained when using the carrier system viral samples as inocula. Instead, the viral yields varied directly with the input when CV-1 cells were infected with carrier system stocks (from weeks 12 and 21) over the range of dilutions examined (undiluted to $\times 20$). Furthermore, defective interfering particle activity, as measured by yield reduction assays (2, 33), was also not detected. In that assay viral yields were titered 72 h after coinfecting CV-1 cells with a constant low input of wild-type SV40 (0.01 PFU per cell) and twofold serial dilutions of the carrier system stocks. These conditions readily revealed defective interfering particle activity in SV40 stocks when the titer exceeded 10^6 defective interfering particles per ml (33, 34). Nevertheless, defective interfering particle activity was not detected in the SV40-A172 carrier system samples. Viral yields in the coinfected CV-1 cultures were about 5×10^6 PFU/ml, regardless of the input from the carrier stocks. Thus, whereas defective interfering particles may be present in the carrier system below our level of detectability, SV40 persistence in A172 cells does not appear to be mediated by or dependent upon the activity of an excess of variant viral particles.

Interferon. The SV40-A172 system was tested for interferon production by direct challenge with vesicular stomatitis virus (Indiana serotype) at low input multiplicity (2×10^{-4} PFU per cell). The 24-h vesicular stomatitis virus yields were about 500 PFU per infected cell in both carrier system and control A172 cells. Thus, interferon is not a factor in the regulation of this carrier system.

Transformation of the carrier system. As noted above, virtually all cells of the SV40-A172 carrier system produced the SV40 intranuclear T antigen. Furthermore, although the A172 cells were derived from glioblastomas (11), persistent

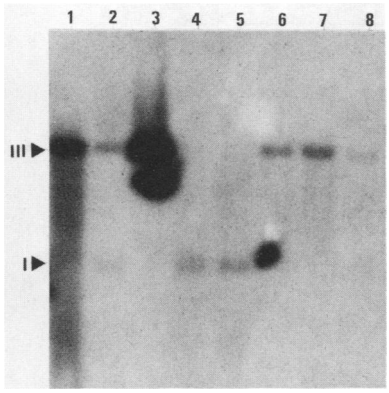

FIG. 2. Southern blot hybridization analysis of viral DNA in the SV40-A172 carrier system. DNA samples from a culture at week 12 of persistent infection were cleaved with restriction endonucleases. The resulting restriction fragments were separated on a 1% agarose gel, blotted onto nitrocellulose filters, and hybridized to ^{32}P-labeled cloned SV40 DNA. Lanes: 1, SV40-A172 Hirt supernatant fraction digested with *BglI*; 2, reconstruction containing a Hirt precipitate of control A172 cells (5 μg) plus wild-type SV40 DNA (4 genome equivalents) digested together with *SstI* and then mixed with *Eco*RI-digested wild-type SV40 DNA (4 genome equivalents); 3, pBR322-cloned SV40 DNA (plasmid P777B) digested with *Bam*HI; 4 through 8, SV40-A172 Hirt precipitate fractions (2.5 μg) either undigested or digested with *SstI*, *Eco*RI, *BglI*, and *Bam*HI, respectively. The positions in the gel of SV40 DNA forms I and III are indicated.

infection also led to changes in cellular growth (relative to the control cultures) characteristic of SV40 transformation. These changes included growth to higher saturation density and an enhanced ability to grow and form colonies when plated at low cell density (Fig. 3). However, neither the growth nor the cloning efficiency of either the persistently infected or control uninfected A172 cultures was affected by reduced serum concentrations. Also note that persistent infection did not impair the ability of the A172 cells to extend processes that establish intercellular contacts (Fig. 4).

Cytogenetic damage induced by SV40 persistence. The karyotypes of the persistently infected and control A172 cultures were examined between passages 41 through 46 and 39 through 44, respectively, from when the original explant culture was initiated. This corresponded to weeks 21 through 24 of persistent infection.

Fifty metaphases from both the uninfected control and persistently infected cultures were analyzed; three of the 50 uninfected cell metaphases displayed chromosomal changes, whereas 18 of the 50 metaphases from the persistently infected culture displayed changes (Fig. 5). These cytogenetic effects included SCE (Fig. 5B and C) and also ring chromosomes (Fig. 5B), acentric fragments (Fig. 5C), and breaks and gaps (Fig. 5D). The distribution of SCE per chromosome in the persistently infected and control cultures is shown in Fig. 6. Persistent infection also caused an increase in the chromosome number (Fig. 7).

DISCUSSION

Restriction of viral replication to only a small portion of the cell population, as found in the SV40-A172 carrier system, is generally necessary for the maintenance of persistent infections involving cytopathic viruses, since it insures the continued survival of a subfraction of potentially susceptible cells. Such restricted viral replication is the major characteristic that distinguishes carrier systems from persistent infections of the steady-state type in which all cells may be productively infected. Steady-state infections generally involve noncytopathic budding RNA viruses.

The productive infection in a carrier system may be restricted by the action of both viral and cellular factors, including defective viral variants, interferon, and other less understood cellular factors (see reference 35 for a review). The expression and relative importance of these factors vary from one system to another.

The establishment and maintenance of the SV40-A172 carrier system appeared to result primarily from the effect of host cell factors, since there were no detectable changes in the viral population from the input SV40 inoculum, whereas the cells expressed incomplete or transient resistance to SV40. Interferon was not a factor in this system.

A similar importance of cellular factors in the establishment and maintenance of persistent SV40 infections of LLC-MK$_2$ rhesus monkey kidney cells was noted earlier (30, 32). In this regard, both the LLC-MK$_2$ and A172 cell lines plate SV40 inefficiently and subsequently display transient or incomplete resistance to productive infection, and both readily generate stable SV40 carrier systems. In contrast, cultures of green monkey kidney (GMK) cell lines (e.g., BSC-1, CV-1), which are much more susceptible to SV40, become persistently infected only after their nearly complete destruction (25, 34). Also, the "established" SV40-GMK cell carrier systems are subsequently less stable, undergoing periodic episodes of crisis not seen in the A172 and LLC-MK$_2$ carrier systems. Furthermore, the A172 cells display even lower levels of infectability and viral yields when infected with the human BK papovavirus than with SV40, and BKV-A172 carrier systems are established without any apparent cytopathic effects whatsoever (Norkin, unpublished results). Thus, a population of restrictive cells may in general be a major factor in the establishment of stable

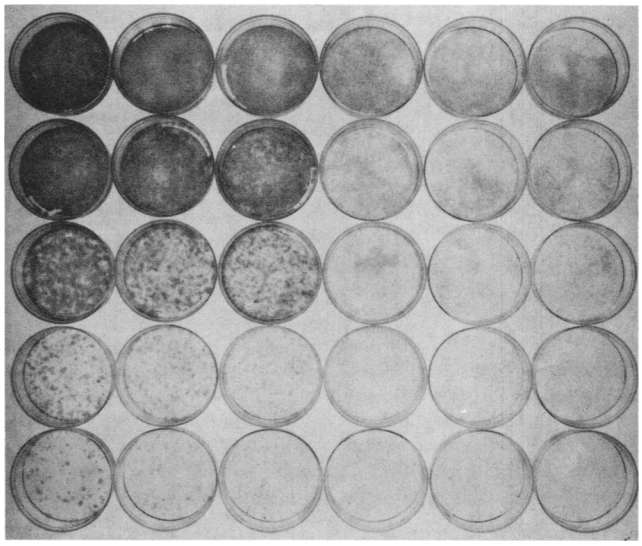

Fig. 3. Effect of persistent infection on colony formation by A172 cells. The plates in columns 1, 2, and 3 were seeded with SV40-infected A172 carrier culture cells in DME medium supplemented with 10, 2.5, and 1% fetal calf serum, respectively. The plates in columns 4, 5, and 6 were seeded with control A172 cells in DME medium supplemented with 10, 2.5, and 1% fetal calf serum, respectively. The plates in rows 1 through 5 received 1.3×10^5, 4.3×10^4, 1.4×10^4, 4.7×10^3, and 1.6×10^3 cells per 60-mm petri dish, respectively. Carrier culture cells were seeded after 11 weeks of persistent infection. All plates were fixed and stained 10 days after plating.

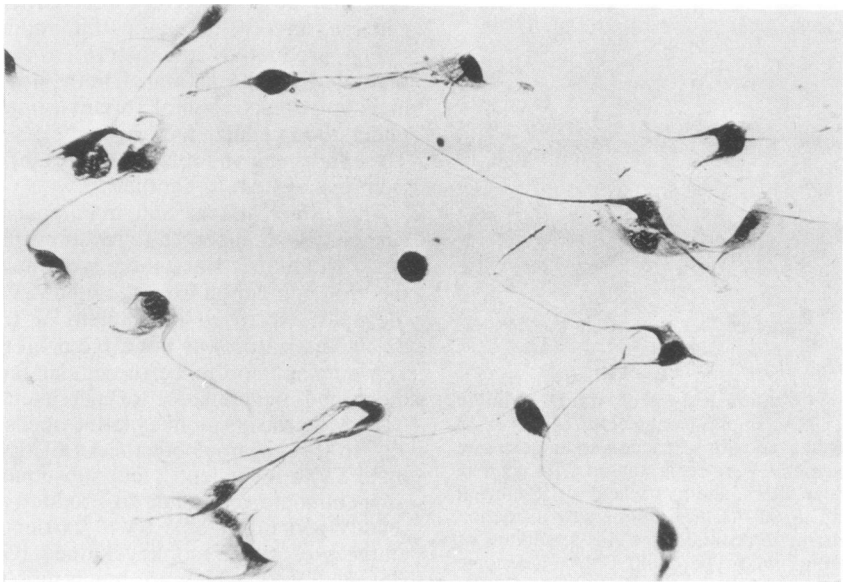

FIG. 4. Light micrograph of Giemsa-stained, SV40-infected A172 cells on day 10 after passage in DME supplemented with 1% fetal calf serum. Note the bipolar glial cell-like morphology, the extent of the processes, and the intercellular contacts. The field is from the plate in column 3, row 5, of Fig. 3.

papovaviral carrier systems. It might be expected that less permissive cell lines might establish carrier systems more readily than more susceptible cell lines because of their ability to restrict viral replication. However, exceptions to this expectation have been observed in other virus-cell systems (18).

Cellular resistance to productive infection in the SV40-A172 system initially reflected, at least in part, the low SV40 plating efficiency on A172 cells, since all of the cells eventually produced the SV40 T antigen. In the established system in which the viral T antigen was produced by all cells, subsequent steps in viral replication must also have been restricted. Whereas the initial infection might have selected for classes of somewhat more resistent cells, resistance had to be incomplete or transient in at least some cells to allow for the continual appearance of new productive cells that perpetuated the productive infection.

The nature of the block in the nonproductive T antigen-positive cells is not yet known. It is expressed early, as indicated by the low average number of viral genomes per cell and by the failure of all but a few cells to make late viral proteins. The block is not explained by failure to make any particular viral protein, since the fraction of cells that immunofluoresce when stained with antisera prepared against disrupted capsids is equivalent to the fraction of cells that react with antisera prepared against purified VP1 or VP3. Note that in most carrier systems involving either RNA or DNA viruses the large majority (and in some cases 100%) of the cells express some viral gene activity as demonstrated by immunofluorescent staining (35, 46).

Because the integration of papovaviral genomes is a well-established phenomenon (51), it is interesting that integrated viral DNA was not detected in the SV40-A172 system. However, integrated viral genomes might have been unrecognized if they were located at a number of different sites in different cells of the system or if they were present in only a small proportion of the cells. In consideration of these possibilities, studies of cloned cell populations are currently in progress. Regardless, other papovaviral carrier systems have been described in which the viral DNA also appeared exclusively in a free state (19, 50). Papovaviral genomes also appear to persist in a free state in vivo, as shown by studies of BK or JC virus DNA in various organs (e.g., kidney, liver, spleen, tonsils, brain) from PML as well as "normal" patients (12, 15, 16). In contrast, integration has been associated with those nonproductive laboratory infections of nonpermissive cells which result in transformation (51). For the maintenance of a carrier system of permissive cells, it would appear to be advantageous for the viral genomes to be in a plasmid state, provided that other mechanisms act to regulate the productive infection.

More enigmatic is the finding that the viral genomes in the SV40-A172 system are homogeneous and nondefective, whereas viral mutants arise, are selected, and act to stabilize other papovaviral carrier systems (24, 33, 34). Indeed, the generation and action of defective viral variants have been advanced as a general mechanism to explain viral persistence and chronic viral disease (20). However, the JC viral DNA isolated directly from the brain of a PML patient is homogeneous (14). In this respect, the SV40-A172 carrier system might reflect an important characteristic of the chronic infection in PML.

Different permissive simian cell lines generate defective SV40, although some do so more readily than others (37). In contrast, in the SV40-glioblastoma carrier system described here and in a persistent infection of human fetal brain cells with BKV (50), episomal viral genomes persisted which were indistinguishable from their respective prototype viral genomes. Nevertheless, neural cells are able to generate defective papovaviral variants. In particular, A172 cells were reported to generate and accumulate defective SV40, even on low-multiplicity viral passes (6). The SV40-A172 carrier system might generate variant viruses at later times. Regardless, viral variants that might have promoted the establishment or early maintenance of this system were not in evidence.

The grossly aberrant nuclei seen in some cells of the SV40-A172 carrier system resemble the giant nuclei seen in

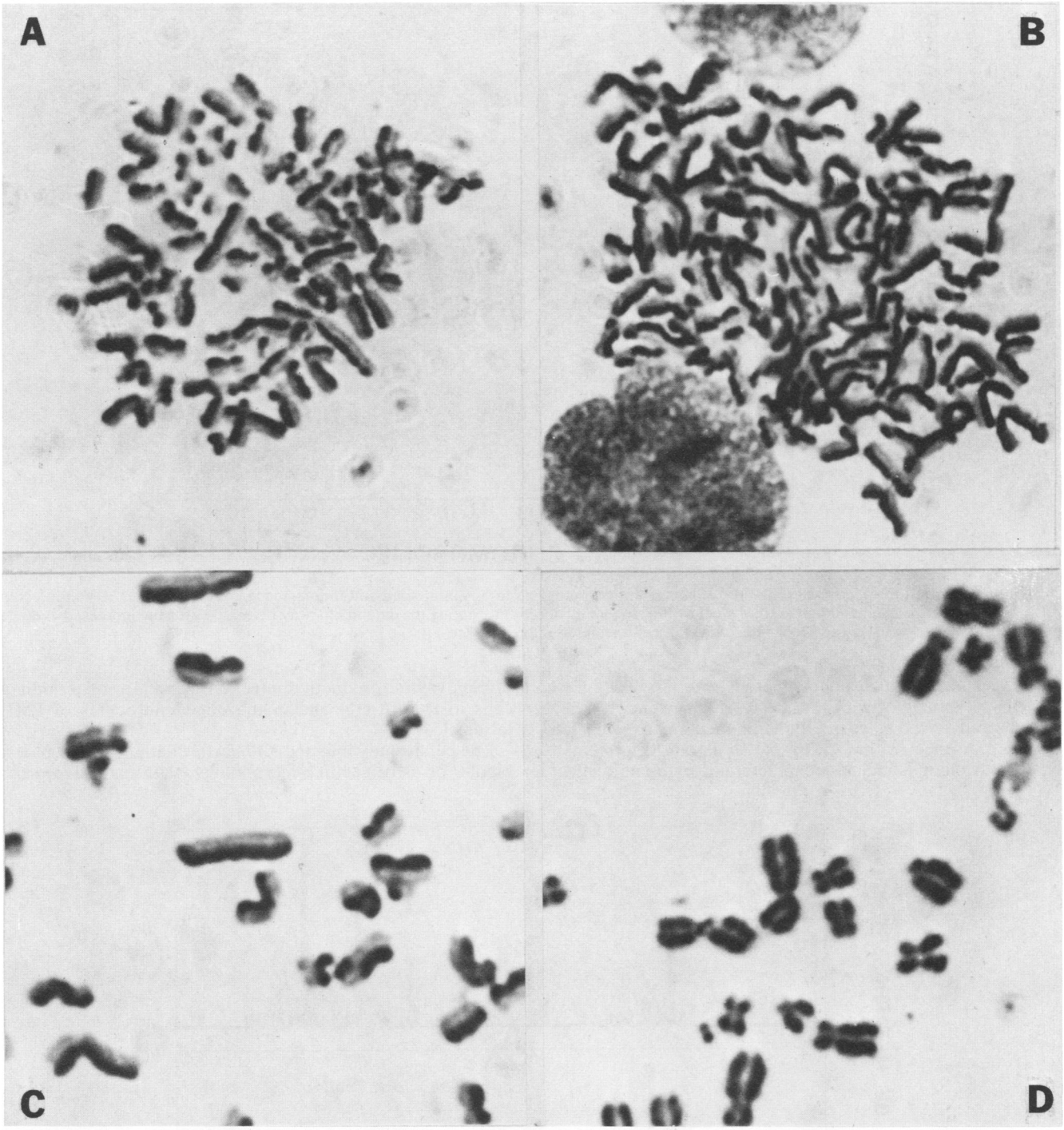

FIG. 5. Chromosomal aberrations and chromatid exchanges in SV40-infected A172 cells after 21 weeks of persistent infection. (A) Uninfected A172 cell metaphase containing about 72 chromosomes. (B) SV40-infected A172 cell metaphase containing about 130 chromosomes and displaying sister chromatid exchanges and a ring chrosome. (C) Portion of SV40-infected A172 cell metaphase showing acentric fragments and a sister chromatid exchange. (D) Portion of SV40-infected A172 cell metaphase showing breaks and gaps.

some astrocytes of PML lesions. Such astrocytes are indeed a distinctive feature of PML. They are otherwise seen only in neoplasms (1), and then only rarely (47). Nevertheless some tumors induced in squirrel and owl monkeys by intracerebral inoculation with JC virus from the brain of a PML patient did contain focal regions of giant cells in which there were huge nuclei (27). These results, and our finding that the aberrant nuclei in the SV40-A172 system correlated with advanced stages of viral gene expression, suggest that papovaviral infection might underlie some human giant cell glioblastomas. However, note that papovavirions are generally not found in the giant astrocytes of PML lesions (53). Also, despite the oncogenic potential of JC virus (38), the neoplastic-like astrocytes in brains of PML patients only

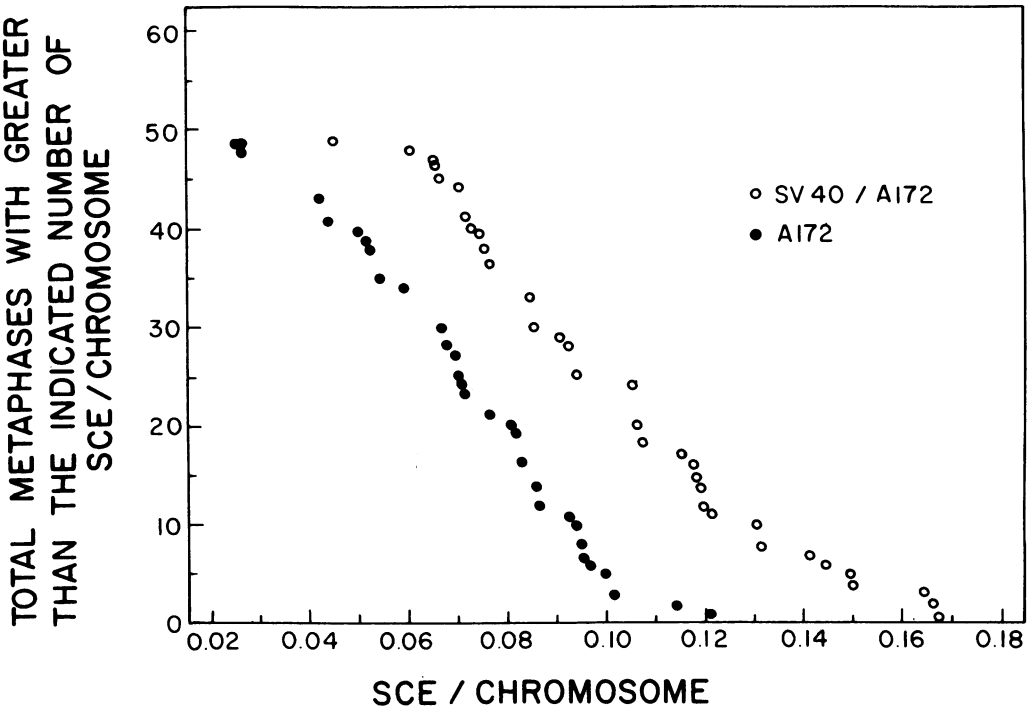

FIG. 6. Distribution of sister chromatid exchanges per chromosome in SV40-infected and uninfected A172 cell cultures. SV40-infected A172 cultures were examined after 21 to 24 weeks of persistent infection. Presenting the data in this way normalizes for the increased average number of chromosomes in the SV40-infected A172 cell metaphase spreads (see Fig. 7).

rarely generate actual tumor masses (7, 48). The giant nucleus-containing cells of the SV40-A172 system probably reflect an advanced stage of lytic viral infection (as indicated by the presence within them of viral capsid proteins). Nuclear swelling is indeed a characteristic cytopathic effect of papovaviral productive infection in both primate kidney cells in culture (10) and in the oligodendrocytes of PML lesions (53).

The persistently infected A172 cells continued to display a bipolar or astrocyte-like morphology, with extensive proc-

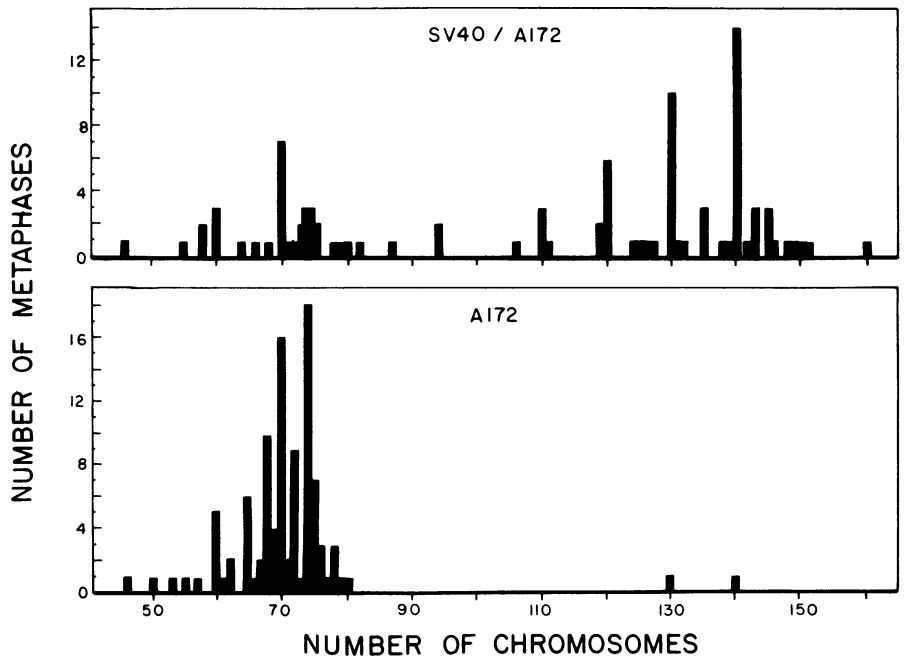

FIG. 7. Distribution of number of chromosomes in different metaphase spreads of SV40-infected and uninfected A172 cells. SV40-infected A172 cells were examined at 21 to 24 weeks of persistent infection.

ess formation and intercellular contacts, similar to that seen in normal brain astrocytes. The retention of glial morphology is noteworthy since it suggests that at least some of the specialized functions of the non-productively infected carrier cells are not necessarily impaired, despite the expression by those cells of the SV40 T antigen and the transformed phenotype. Further studies on the effects of SV40 persistence on the specialized glial functions of these cells are in progress.

The SV40-A172 carrier system reflects aspects of the interaction of papovaviruses with both oligodendrocytes and astrocytes in the brains of PML patients. Oligodendrocytes are the only cells that appear to be productively infected in PML, with resultant oligodendrocytopathy (9, 26). In contrast, astrocytes do not appear to be productively infected, but (as noted above) they may exhibit extraordinarily deformed nuclei and a neoplastic appearance. The A172 line of glioblastoma cells is probably astrocytic in origin. However, other clonal cell lines derived from glioblastomas indeed express combinations of oligodendroglial and astrocytic markers. This might reflect the likelihood that astrocytes and oligodendrocytes are derived from a common glial cell precursor (44).

Because several viruses, including the papovaviruses (4, 29), induce chromosomal alterations, we were interested in the effects of persistent SV40 infection on the karyotype of the A172 human neural cells. SV40 persistence indeed caused a variety of chromosomal changes in those cells. Included among these were SCE, which are believed to result from a DNA repair process since their incidence is enhanced by drugs or treatments (e.g., UV, alkylating agents) that induce lesions that appear after DNA replication (5, 22).

Studies of rat fibroblasts, transformed by a polyomavirus mutant temperature sensitive for the viral T antigen function, showed that an increase in SCE occurred only under conditions in which T antigen was active (4). In that system, SCE were promoted by the activity of integrated papovaviral genomes. The results reported here suggest that SCE can also be induced by the activity of free papovaviral genomes. This is of interest in comparison to retrovirus-induced SCE, which does not occur in cells in which integration is prevented by host cell restriction (42), suggesting that integration may underlie retrovirus-induced SCE. In contrast, papovavirus-induced SCE would appear to result from a direct or indirect effect of T-antigen expression.

It is not yet known what relationship, if any, exists between the induction of these cytogenetic effects and the transformation of the SV40-A172 system. However, note that chromosomal changes are induced by nononcogenic, as well as oncogenic, viruses (28).

ACKNOWLEDGMENTS

We thank Frank Smiddy for his excellent technical assistance and Donna Tudryn for her expert preparation of the manuscript. We are grateful to Frank O'Neill for the A172 cells, Harvey Ozer and Harumi Kasamutsu for antisera, and Michael Piatak for cloned SV40 DNA.

This work was supported by Public Health Service grant AI-14049 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Astrom, K. E., E. L. Mancall, and E. P. Richardson.** 1958. Progressive multifocal leukoencephalopathy. Brain **81**:93–111.
2. **Bellett, A. J. D., and P. O. Copper.** 1959. Some properties of the transmissible interfering component of VSV preparation. Gen. Microbiol. **21**:498–509.
3. **Botchan, M., W. Topp, and J. Sambrook.** 1976. The arrangement of simian virus 40 sequences in the DNA of transformed cells. Cell **9**:269–287.
4. **Brown, E. H., and C. Basilico.** 1982. Induction of sister chromatid exchange by polyma large viral tumor antigen in transformed rat fibroblasts. Cancer Res. **42**:1409–1912.
5. **Carrano, A. V., L. H. Thompson, P. A. Lindl, and J. L. Minkler.** 1978. Sister chromatid exchange as an indicator of mutagenesis. Nature (London) **271**:551–553.
6. **Carroll, D., J. L. Hanson, E. B. Maryon, and F. J. O'Neill.** 1981. SV40 defectives selected during low multiplicity passage on A172 human glioblastoma cells. Virology **112**:461–471.
7. **Castaigne, P., P. Roudot, R. Escourolle, J. L. Ribadeau-Dumas, F. Cathala, and J. J. Hauw.** 1974. Leukoencephalopathic multifocale progressive et "gliomes" multiple. Rev. Neurol. (Paris) **130**:379–392.
8. **Davoli, D., D. Ganem, A. L. Nussbaum, G. C. Fareed, P. M. Howley, G. Khoury, and M. Martin.** 1977. Genome structures of reiteration mutants of simian virus 40. Virology **77**:110–124.
9. **Dorries, K., R. T. Johnson, and V. ter Meulen.** 1979. Detection of polyoma virus DNA in PML-brain tissue by (*in situ*) hybridization. J. Gen. Virol. **42**:49–57.
10. **Einck, K. H., and L. C. Norkin.** 1979. Lysosome stability during lytic infection by simian virus 40. Intervirology **12**:47–56.
11. **Giard, D. J., S. A. Aaronson, G. T. Todaro, P. Arnstein, J. H. Kersey, H. Dostik, and W. P. Parks.** 1973. In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J. Natl. Cancer Inst. **51**:1417–1422.
12. **Goudsmit, J., P. Wertheim-Van Dillen, and A. Van-Strien, and J. Van Der Noordaa.** 1982. The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. J. Med. Virol. **10**:91–99.
13. **Gribble, D. H., C. C. Haden, L. W. Schwartz, and R. V. Hendrickson.** 1975. Spontaneous progressive multifocal leukoencephalopathy (PML) in macaques. Nature (London) **254**: 602–604.
14. **Grinnell, B. W., J. D. Martin, B. L. Pagdett, and D. L. Walker.** 1982. Is progressive multifocal leukoencephalopathy a chronic disease because of defective interfering particles or temperature-sensitive mutants of JC virus? J. Virol. **43**:1143–1150.
15. **Grinnell, B. W., B. L. Pagdett, and D. L. Walker.** 1983. Distribution of nonintegrated DNA from JC papovavirus in organs of patients with progressive multifocal leukoencephalopathy. Infect. Dis. **147**:669–675.
16. **Heritage, J., P. M. Chesters, and D. J. McCance.** 1981. The persistence of papovavirus BK DNA sequences in normal human kidney. J. Med. Virol. **8**:143–150.
17. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
18. **Holland, J. J., L. P. Villareal, R. M. Welsh, M. B. A. Oldstone, D. Kohne, R. Lazzarini, and E. Scolnick.** 1976. Long term persistent vesicular stomatitis virus and rabies virus infection of cells in vitro. J. Gen. Virol. **33**:193–211.
19. **Howley, P. M., F. Rentier-Delrue, L. A. Heilman, M. F. Law, K. Chowdhury, M. A. Israel, and K. K. Takemoto.** 1980. Cloned human polyomavirus JC DNA can transform human amnion cells. J. Virol. **36**:878–882.
20. **Huang, A. S., and D. Baltimore.** 1970. Defective viral particles and viral disease processes. Nature (London) **226**:323–327.
21. **Ketner, G., and T. G. Kelly.** 1976. Integrated simian virus 40 sequences in transformed cell DNA: analysis using restriction endonucleases. Proc. Natl. Acad. Sci. U.S.A. **73**:1102–1106.
22. **Kihlman, B. A.** 1975. Sister chromatid exchanges in *Vicia faba*. II. Effects of thiotepa, caffeine, and 8-ethoxycaffeine on the frequency of SCE. Chromosome **52**:11–18.
23. **Lee, T. N. H., W. W. Brockman, and D. Nathans.** 1975. Evolutionary variants of simian virus 40: cloned substituted variants containing multiple initiation sites for DNA replication. Virology **66**:53–69.
24. **Lombardi, P. S., P. Balduzzi, J. D. Hare, and H. R. Morgan.** 1970. Mechanism of the development of a stable carrier system of L cells with polyoma virus. J. Natl. Cancer Inst. **45**:171–178.

25. **Margalith M., R. Volk-Fuchs, and N. Goldblum.** 1969. Transformation of BSC-1 cells following chronic infection with SV40. J. Gen. Virol. **5**:321–327.
26. **Mazlo, M., and R. M. Herndon.** 1977. Progressive multifocal leukoencephalopathy: ultrastructural findings in two brain biopsies. Neuropathol. Appl. Neurobiol. **3**:323–339.
27. **McKeever, P. E., B. M. Chronwall, S. A. Houff, J. L. Sever, P. L. Kornblith, B. L. Padgett, D. L. Walker, and W. T. London.** 1983. Glial and divergent cells in primate central nervous system tumors induced by JC virus isolated from human progressive multifocal leukoencephalopathy, p. 239–251. *In* J. L. Sever and D. L. Madden (ed.), Polyomaviruses and human neurological diseases. Alan R. Liss, New York.
28. **Nichols, W. W.** 1983. Viral interactions with the mammalian genome relevant to neoplasia, p. 317–332. *In* J. Germen (ed.), Chromosome mutation and neoplasia. Alan R. Liss, New York.
29. **Nichols, W. W., C. I. Bradt, L. H. Toji, M. Godley, and M. Segawa.** 1978. Induction of sister chromatid exchanges by transformation with simian virus 40. Cancer Res. **38**:960–964.
30. **Norkin, L. C.** 1976. Rhesus monkey kidney cells persistently infected with simian virus 40: production of defective interfering virus and acquisition of the transformed phenotype. Infect. Immun. **14**:783–792.
31. **Norkin, L. C.** 1977. Cell killing by simian virus 40: impairment of membrane formation and function. J. Virol. **21**:872–879.
32. **Norkin, L. C.** 1977. Effect of input multiplicity on the establishment of simian virus 40 persistent infections in rhesus monkey kidney cells. Infect. Immun. **18**:868–871.
33. **Norkin, L. C.** 1979. The emergences of SV40 variants in a persistent infection of rhesus monkey kidney cells and their interactions with standard simian virus 40. Virology **95**:598–603.
34. **Norkin, L. C.** 1980. Persistent infections of green monkey kidney cells established with temperature-sensitive mutants of simian virus 40. Virology **107**:375–388.
35. **Norkin, L. C.** 1982. Papovaviral persistent infections. Microbiol. Rev. **46**:384–425.
36. **Norkin, L. C., and J. Ouellette.** 1976. Cell killing by simian virus 40: variation in the pattern of lysosomal enzyme release cellular enzyme release, and cell death during productive infection of normal and simian virus 40-transformed simian cell lines. J. Virol. **18**:48–57.
37. **Norkin, L. C., J. B. Wojcik, and C. A. Goguen.** 1981. Effect of the host cell on the generation of defective simian virus 40 during undiluted serial passages and persistent infection. Virology **108**:525–530.
38. **Padgett, B.** 1980. Human papovaviruses, p. 339–370. *In* J. Tooze (ed.), DNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
39. **Padgett, B. L., C. M. Rogers, and D. L. Walker.** 1977. JC virus, a human polyomavirus associated with progressive multifocal leukoencephalopathy: additional biological characteristics and antigenic relationships. Infect. Immun. **15**:656–662.
40. **Padgett, B. L., and D. L. Walker.** 1980. Human papovavirus JCV: natural history, tumorigenicity, and interaction with human cells in culture, p. 319–327. *In* M. Essex, G. Todaro, and H. zur Haussen (ed.), Viruses in naturally occurring cancers. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
41. **Padgett, B. L., D. L. Walker, G. M. ZuRhein, and J. M. Varakis.** 1977. Differential neurooncogenicity of strains of JC virus, a human polyomavirus, in newborn Syrian hamsters. Cancer Res. **37**:718–720.
42. **Palitti, F., G. P. Matarese, G. Diana, V. Sorrentino, and G. B. Rossi.** 1982. *In vivo* studies of increased incidence of sister chromatid exchanges in target cells of replication-competent Friend murine leukemia virus. Cancer Res. **42**:4753–4757.
43. **Perry, P., and S. Wolff.** 1974. New Giemsa method for the differential staining of sister chromatids. Nature (London) **251**:156–158.
44. **Raff, M. C., R. H. Miller, and M. Noble.** 1983. A glial progenitor cell that develops *in vitro* into an astrocyte or an oligodendrocyte depending on the culture medium. Nature (London) **303**:390–396.
45. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
46. **Rima, B. K., and S. J. Martin.** 1976. Persistent infection of tissue culture cells by RNA viruses. Med. Microbiol. Immunol. **162**:89–118.
47. **Rubinstein, L. J.** 1972. Tumors of the central nervous system, p. 42–84. *In* Atlas of tumor pathology, second series Armed Forces Institute of Pathology, Washington, D.C.
48. **Sima, A. A. F., S. D. Finkelstein, and D. R. McLachlan.** 1983. Multiple malignant astrocytomas in a patient with spontaneous progressive multifocal leukoencephalopathy. Ann. Neurol. **14**:183–188.
49. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
50. **Takemoto, K. K., H. Linke, T. Miyamura, and G. C. Fareed.** 1979. Persistent BK papovavirus infection of transformed human fetal brain cell. I. Episomal viral DNA in cloned lines deficient in T-antigen expression. J. Virol. **12**:625–631.
51. **Tooze, J.** (ed.). 1980. Molecular biology of tumor viruses. II. DNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
52. **Weiner, L. P., R. M. Herndon, O. Narayan, R. T. Johnson, K. Shah, L. J. Rubenstein, T. J. Preziosi, and F. K. Conley.** 1972. Isolation of virus related to SV40 from patients with progressive multifocal leukoencephalopathy. N. Engl. J. Med. **286**:385–390.
53. **Zu Rhein, G. M.** 1969. Association of papova virions with human demyelinating disease (progressive multifocal leukoencephalopathy). Prog. Med. Virol. **11**:185–247.
54. **Zu Rhein, G. M., and S. M. Chou.** 1965. Particles resembling papova viruses in human cerebral demyelinating disease. Science **148**:1477–1479.